

**PCT**WORLD INTELLECTUAL PROPERTY ORGANIZATION  
International Bureau

## INTERNATIONAL APPLICATION PUBLISHED UNDER THE PATENT COOPERATION TREATY (PCT)

<b>(51) International Patent Classification <sup>6</sup>:</b> <b>C12N 9/12, C12Q 1/48</b>	<b>A1</b>	<b>(11) International Publication Number:</b> <b>WO 99/01541</b> <b>(43) International Publication Date:</b> 14 January 1999 (14.01.99)
<b>(21) International Application Number:</b> PCT/US98/13782 <b>(22) International Filing Date:</b> 1 July 1998 (01.07.98)  <b>(30) Priority Data:</b> 08/887,115 1 July 1997 (01.07.97) US 08/890,854 10 July 1997 (10.07.97) US  <b>(71) Applicant:</b> TULARIK INC. [US/US]; Two Corporate Drive, South San Francisco, CA 94080 (US).  <b>(72) Inventors:</b> ROTH, Mike; Tularik Inc., Two Corporate Drive, South San Francisco, CA 94080 (US). CAO, Zhaodan; Tularik Inc., Two Corporate Drive, South San Francisco, CA 94080 (US). REGNIER, Catherine; Tularik Inc., Two Corporate Drive, South San Francisco, CA 94080 (US).  <b>(74) Agent:</b> OSMAN, Richard, Aron; Science & Technology Law Group, 75 Denise Drive, Hillsborough, CA 94010 (US).		<b>(81) Designated States:</b> AL, AM, AT, AU, AZ, BA, BB, BG, BR, BY, CA, CH, CN, CU, CZ, DE, DK, EE, ES, FI, GB, GE, GH, GM, GW, HR, HU, IL, IS, JP, KE, KG, KP, KR, KZ, LC, LK, LR, LS, LT, LU, LV, MD, MG, MK, MN, MW, MX, NO, NZ, PL, PT, RO, RU, SD, SE, SG, SI, SK, SL, TJ, TM, TR, TT, UA, UG, UZ, VN, YU, ZW, ARIPO patent (GH, GM, KE, LS, MW, SD, SZ, UG, ZW), Eurasian patent (AM, AZ, BY, KG, KZ, MD, RU, TJ, TM), European patent (AT, BE, CH, CY, DE, DK, ES, FI, FR, GB, GR, IE, IT, LU, MC, NL, PT, SE), OAPI patent (BF, BJ, CF, CG, CI, CM, GA, GN, ML, MR, NE, SN, TD, TG).  <b>Published</b> <i>With international search report.</i>
<b>(54) Title:</b> IKK- $\alpha$ PROTEINS, NUCLEIC ACIDS AND METHODS  <b>(57) Abstract</b>  The invention provides methods and compositions relating to an I $\kappa$ B kinase, IKK- $\alpha$ , and related nucleic acids. The polypeptides may be produced recombinantly from transformed host cells from the disclosed IKK- $\alpha$ encoding nucleic acids or purified from human cells. The invention provides isolated IKK- $\alpha$ hybridization probes and primers capable of specifically hybridizing with the disclosed IKK- $\alpha$ genes, IKK- $\alpha$ -specific binding agents such as specific antibodies, and methods of making and using the subject compositions in diagnosis, therapy and in the biopharmaceutical industry.		

**FOR THE PURPOSES OF INFORMATION ONLY**

Codes used to identify States party to the PCT on the front pages of pamphlets publishing international applications under the PCT.

AL	Albania	ES	Spain	LS	Lesotho	SI	Slovenia
AM	Armenia	FI	Finland	LT	Lithuania	SK	Slovakia
AT	Austria	FR	France	LU	Luxembourg	SN	Senegal
AU	Australia	GA	Gabon	LV	Latvia	SZ	Swaziland
AZ	Azerbaijan	GB	United Kingdom	MC	Monaco	TD	Chad
BA	Bosnia and Herzegovina	GE	Georgia	MD	Republic of Moldova	TG	Togo
BB	Barbados	GH	Ghana	MG	Madagascar	TJ	Tajikistan
BE	Belgium	GN	Guinea	MK	The former Yugoslav	TM	Turkmenistan
BF	Burkina Faso	GR	Greece		Republic of Macedonia	TR	Turkey
BG	Bulgaria	HU	Hungary	ML	Mali	TT	Trinidad and Tobago
BJ	Benin	IE	Ireland	MN	Mongolia	UA	Ukraine
BR	Brazil	IL	Israel	MR	Mauritania	UG	Uganda
BY	Belarus	IS	Iceland	MW	Malawi	US	United States of America
CA	Canada	IT	Italy	MX	Mexico	UZ	Uzbekistan
CF	Central African Republic	JP	Japan	NE	Niger	VN	Viet Nam
CG	Congo	KE	Kenya	NL	Netherlands	YU	Yugoslavia
CH	Switzerland	KG	Kyrgyzstan	NO	Norway	ZW	Zimbabwe
CI	Côte d'Ivoire	KP	Democratic People's	NZ	New Zealand		
CM	Cameroon		Republic of Korea	PL	Poland		
CN	China	KR	Republic of Korea	PT	Portugal		
CU	Cuba	KZ	Kazakhstan	RO	Romania		
CZ	Czech Republic	LC	Saint Lucia	RU	Russian Federation		
DE	Germany	LI	Liechtenstein	SD	Sudan		
DK	Denmark	LK	Sri Lanka	SE	Sweden		
EE	Estonia	LR	Liberia	SG	Singapore		

*IKK- $\alpha$  Proteins, Nucleic Acids and Methods*

## INTRODUCTION

Field of the Invention

The field of this invention is proteins involved in transcription factor activation.

Background

Cytokines trigger changes in gene expression by modifying the activity of otherwise latent transcription factors (Hill and Treisman, 1995). Nuclear factor  $\kappa$ B (NF- $\kappa$ B) is a prominent example of how such an external stimulus is converted into an active transcription factor (Verma et al., 1995). The NF- $\kappa$ B system is composed of homo- and heterodimers of members of the Rel family of related transcription factors that control the expression of numerous immune and inflammatory response genes as well as important viral genes (Lenardo and Baltimore, 1989; Baeuerle and Henkel, 1994). The activity of NF- $\kappa$ B transcription factors is regulated by their subcellular localization (Verma et al., 1995). In most cell types, NF- $\kappa$ B is present as a heterodimer comprising of a 50 kDa and a 65 kDa subunit. This heterodimer is sequestered in the cytoplasm in association with I $\kappa$ B $\alpha$  a member of the I $\kappa$ B family of inhibitory proteins (Finco and Baldwin, 1995; Thanos and Maniatis, 1995; Verma et al., 1995). I $\kappa$ B $\alpha$  masks the nuclear localization signal of NF- $\kappa$ B and thereby prevents NF- $\kappa$ B nuclear translocation. Conversion of NF- $\kappa$ B into an active transcription factor that translocates into the nucleus and binds to cognate DNA sequences requires the phosphorylation and subsequent ubiquitin-dependent degradation of I $\kappa$ B $\alpha$  in the 26s proteasome. Signal-induced phosphorylation of I $\kappa$ B $\alpha$  occurs at serines 32 and 36. Mutation of one or both of these serines renders I $\kappa$ B $\alpha$  resistant to ubiquitination and proteolytic degradation (Chen et al., 1995).

The pleiotropic cytokines tumor necrosis factor (TNF) and interleukin-1 (IL-1) are among the physiological inducers of I $\kappa$ B phosphorylation and subsequent NF- $\kappa$ B activation (Osborn et al., 1989; Beg et al., 1993). Although TNF and IL-1 initiate signaling cascades leading to NF- $\kappa$ B activation via distinct families of cell-surface receptors (Smith et al., 1994; Dinarello, 1996), both pathways utilize members of the TNF receptor-associated factor (TRAF) family of adaptor proteins as signal transducers (Rothe et al., 1995; Hsu et al., 1996; Cao et al., 1996b). TRAF proteins were originally found to

associate directly with the cytoplasmic domains of several members of the TNF receptor family including the 75 kDa TNF receptor (TNFR2), CD40, CD30, and the lymphotoxin- $\beta$  receptor (Rothe et al., 1994; Hu et al., 1994; Cheng et al., 1995; Mosialos et al., 1995; Song and Donner, 1995; Sato et al., 1995; Lee et al., 1996; Gedrich et al., 1996; Ansseau et al., 1996). In addition, TRAF proteins are recruited indirectly to the 55 kDa TNF receptor (TNFR1) by the adaptor protein TRADD (Hsu et al., 1996). Activation of NF- $\kappa$ B by TNF requires TRAF2 (Rothe et al., 1995; Hsu et al., 1996). TRAF5 has also been implicated in NF- $\kappa$ B activation by members of the TNF receptor family (Nakano et al., 1996). In contrast, TRAF6 participates in NF- $\kappa$ B activation by IL-1 (Cao et al., 1996b). Upon IL-1 treatment, TRAF6 associates with IRAK, a serine-threonine kinase that binds to the IL-1 receptor complex (Cao et al., 1996a).

The NF- $\kappa$ B-inducing kinase (NIK) is a member of the MAP kinase kinase kinase (MAP3K) family that was identified as a TRAF2-interacting protein (Malinin et al., 1997). NIK activates NF- $\kappa$ B when overexpressed, and kinase-inactive mutants of NIK comprising its TRAF2-interacting C-terminal domain (NIK<sub>(624-947)</sub>) or lacking two crucial lysine residues in its kinase domain (NIK<sub>(KK429-430AA)</sub>) behave as dominant-negative inhibitors that suppress TNF-, IL-1-, and TRAF2-induced NF- $\kappa$ B activation (Malinin et al., 1997). Recently, NIK was found to associate with additional members of the TRAF family, including TRAF5 and TRAF6. Catalytically inactive mutants of NIK also inhibited TRAF5- and TRAF6-induced NF- $\kappa$ B activation, thus providing a unifying concept for NIK as a common mediator in the NF- $\kappa$ B signaling cascades triggered by TNF and IL-1 downstream of TRAFs.

Here, we disclose a novel human kinase I $\kappa$ B Kinase, IKK- $\alpha$ , as a NIK-interacting protein. IKK- $\alpha$  has sequence similarity to the conceptual translate of a previously identified open reading frame (SEQ ID NO:5) postulated to encode a serine-threonine kinase of unknown function ('Conserved Helix-loop-helix Ubiquitous Kinase' or CHUK, Connelly and Marcu, 1995; Mock et al., 1995). Catalytically inactive mutants of IKK- $\alpha$  are shown to suppress NF- $\kappa$ B activation induced by TNF and IL-1 stimulation as well as by TRAF and NIK overexpression; transiently expressed IKK- $\alpha$  is shown to associate with the endogenous I $\kappa$ B $\alpha$  complex; and IKK- $\alpha$  is shown to phosphorylate I $\kappa$ B $\alpha$  on serines 32 and 36.

## SUMMARY OF THE INVENTION

The invention provides methods and compositions relating to isolated IKK- $\alpha$  polypeptides, related nucleic acids, polypeptide domains thereof having IKK- $\alpha$ -specific structure and activity and modulators of IKK- $\alpha$  function, particularly I $\kappa$ B kinase activity. IKK- $\alpha$  polypeptides can regulate NF $\kappa$ B activation and hence provide important regulators of cell function. The polypeptides may be produced recombinantly from transformed host cells from the subject IKK- $\alpha$  polypeptide encoding nucleic acids or purified from mammalian cells. The invention provides isolated IKK- $\alpha$  hybridization probes and primers capable of specifically hybridizing with the disclosed IKK- $\alpha$  gene, IKK- $\alpha$ -specific binding agents such as specific antibodies, and methods of making and using the subject compositions in diagnosis (e.g. genetic hybridization screens for IKK- $\alpha$  transcripts), therapy (e.g. IKK- $\alpha$  kinase inhibitors to inhibit TNF signal transduction) and in the biopharmaceutical industry (e.g. as immunogens, reagents for isolating other transcriptional regulators, reagents for screening chemical libraries for lead pharmacological agents, etc.).

## DETAILED DESCRIPTION OF THE INVENTION

The nucleotide sequence of a natural cDNA encoding a human IKK- $\alpha$  polypeptide is shown as SEQ ID NO:3, and the full conceptual translate is shown as SEQ ID NO:4. The IKK- $\alpha$  polypeptides of the invention include incomplete translates of SEQ ID NO:3, particularly of SEQ ID NO:3, residues 1-638, which translates and deletion mutants of SEQ ID NO:4 have human IKK- $\alpha$ -specific amino acid sequence, binding specificity or function and comprise at least one of Cys30, GluLeu604, Thr679, Ser680, Pro684, Thr686, and Ser678. Preferred translates/deletion mutants comprise at least a 6 residue Cys30, Glu543, Leu604, Thr679, Ser680, Pro684, Thr686 or Ser687-containing domain of SEQ ID NO:4, preferably including at least 8, more preferably at least 12, most preferably at least 20 contiguous residues which immediately flank said residue, with said residue preferably residing within said contiguous residues, see, e.g. Table I, which mutants provide hIKK- $\alpha$  specific epitopes and immunogens.

TABLE 1. Exemplary IKK- $\alpha$  polypeptides having IKK- $\alpha$  binding specificity

hIKK- $\alpha\Delta 1$  (SEQ ID NO:4, residues 1-30) hIKK- $\alpha\Delta 1$  (SEQ ID NO:4, residues 686-699)  
hIKK- $\alpha\Delta 1$  (SEQ ID NO:4, residues 22-31) hIKK- $\alpha\Delta 1$  (SEQ ID NO:4, residues 312-345)  
hIKK- $\alpha\Delta 1$  (SEQ ID NO:4, residues 599-608) hIKK- $\alpha\Delta 1$  (SEQ ID NO:4, residues 419-444)  
hIKK- $\alpha\Delta 1$  (SEQ ID NO:4, residues 601-681) hIKK- $\alpha\Delta 1$  (SEQ ID NO:4, residues 495-503)  
5 hIKK- $\alpha\Delta 1$  (SEQ ID NO:4, residues 604-679) hIKK- $\alpha\Delta 1$  (SEQ ID NO:4, residues 565-590)  
hIKK- $\alpha\Delta 1$  (SEQ ID NO:4, residues 670-687) hIKK- $\alpha\Delta 1$  (SEQ ID NO:4, residues 610-627)  
hIKK- $\alpha\Delta 1$  (SEQ ID NO:4, residues 679-687) hIKK- $\alpha\Delta 1$  (SEQ ID NO:4, residues 627-638)  
hIKK- $\alpha\Delta 1$  (SEQ ID NO:4, residues 680-690) hIKK- $\alpha\Delta 1$  (SEQ ID NO:4, residues 715-740)  
hIKK- $\alpha\Delta 1$  (SEQ ID NO:4, residues 684-695) hIKK- $\alpha\Delta 1$  (SEQ ID NO:4, residues 737-745)

10 The subject domains provide IKK- $\alpha$  domain specific activity or function, such as  
IKK- $\alpha$ -specific kinase or kinase inhibitory activity, NIK-binding or binding inhibitory  
activity, I $\kappa$ B-binding or binding inhibitory activity, NF $\kappa$ B activating or inhibitory activity  
or antibody binding. Preferred domains phosphorylate at least one and preferably both the  
serine 32 and 36 of I $\kappa$ B (Verma, I. M., et al. (1995)). As used herein, Ser32 and Ser36 of  
15 I $\kappa$ B refers collectively to the two serine residues which are part of the consensus sequence  
DSGL/TXSM/L (e.g. ser 32 and 36 in I $\kappa$ B $\alpha$ , ser 19 and 23 in I $\kappa$ B $\beta$ , and ser 157 and 161,  
or 18 and 22, depending on the usage of methionines, in I $\kappa$ B $\epsilon$ , respectively.

IKK- $\alpha$ -specific activity or function may be determined by convenient *in vitro*, cell-  
based, or *in vivo* assays: e.g. *in vitro* binding assays, cell culture assays, in animals (e.g.  
20 gene therapy, transgenics, etc.), etc. Binding assays encompass any assay where the  
molecular interaction of an IKK- $\alpha$  polypeptide with a binding target is evaluated. The  
binding target may be a natural intracellular binding target such as an IKK- $\alpha$  substrate, a  
IKK- $\alpha$  regulating protein or other regulator that directly modulates IKK- $\alpha$  activity or its  
localization; or non-natural binding target such as a specific immune protein such as an  
25 antibody, or an IKK- $\alpha$  specific agent such as those identified in screening assays such as  
described below. IKK- $\alpha$ -binding specificity may assayed by kinase activity or binding  
equilibrium constants (usually at least about  $10^7$  M $^{-1}$ , preferably at least about  $10^8$  M $^{-1}$ ,  
more preferably at least about  $10^9$  M $^{-1}$ ), by the ability of the subject polypeptide to function  
as negative mutants in IKK- $\alpha$ -expressing cells, to elicit IKK- $\alpha$  specific antibody in a  
30 heterologous host (e.g. a rodent or rabbit), etc. In any event, the IKK- $\alpha$  binding specificity

of the subject IKK- $\alpha$  polypeptides necessarily distinguishes the murine and human CHUK sequences of Connelly and Marcu (1995) as well as IKK- $\beta$  (SEQ ID NO:4).

The claimed IKK- $\alpha$  polypeptides are isolated or pure: an "isolated" polypeptide is unaccompanied by at least some of the material with which it is associated in its natural state, preferably constituting at least about 0.5%, and more preferably at least about 5% by weight of the total polypeptide in a given sample and a pure polypeptide constitutes at least about 90%, and preferably at least about 99% by weight of the total polypeptide in a given sample. In a particular embodiments, IKK- $\alpha$  polypeptides are isolated from a MKP-1 precipitable complex, isolated from a IKK complex, and/or isolated from IKK- $\beta$ . The IKK- $\alpha$  polypeptides and polypeptide domains may be synthesized, produced by recombinant technology, or purified from mammalian, preferably human cells. A wide variety of molecular and biochemical methods are available for biochemical synthesis, molecular expression and purification of the subject compositions, see e.g. Molecular Cloning, A Laboratory Manual (Sambrook, *et al.* Cold Spring Harbor Laboratory), Current Protocols in Molecular Biology (Eds. Ausubel, *et al.*, Greene Publ. Assoc., Wiley-Interscience, NY) or that are otherwise known in the art.

The invention provides binding agents specific to IKK polypeptides, preferably the claimed IKK- $\alpha$  polypeptides, including substrates, agonists, antagonists, natural intracellular binding targets, etc., methods of identifying and making such agents, and their use in diagnosis, therapy and pharmaceutical development. For example, specific binding agents are useful in a variety of diagnostic and therapeutic applications, especially where disease or disease prognosis is associated with improper utilization of a pathway involving the subject proteins, e.g. NF- $\kappa$ B activation. Novel IKK-specific binding agents include IKK-specific receptors, such as somatically recombined polypeptide receptors like specific antibodies or T-cell antigen receptors (see, e.g. Harlow and Lane (1988) Antibodies, A Laboratory Manual, Cold Spring Harbor Laboratory) and other natural intracellular binding agents identified with assays such as one-, two- and three-hybrid screens, non-natural intracellular binding agents identified in screens of chemical libraries such as described below, etc. Agents of particular interest modulate IKK function, e.g. IKK-dependent transcriptional activation. For example, a wide variety of inhibitors of IKK I $\kappa$ B kinase activity may be used to regulate signal transduction involving I $\kappa$ B. Exemplary IKK I $\kappa$ B kinase inhibitors include known classes of serine/threonine kinase (e.g. PKC)

inhibitors such as competitive inhibitors of ATP and substrate binding, antibiotics, IKK-derived peptide inhibitors, etc., see Tables II and III. IKK specificity and activity are readily quantified in high throughput kinase assays using panels of protein kinases (see cited references and Examples).

Preferred inhibitors include natural compounds such as staurosporine (Omura S, et al. J Antibiot (Tokyo) 1995 Jul;48(7):535-48), produced by a marine organism, and synthetic compounds such as PD 153035, which also potentially inhibits the EGF receptor protein kinase (Fry DW et al. Science 1994 Aug 19;265(5175):1093-5). Members of the tyrphostin family of synthetic protein kinase inhibitors are also useful; these include compounds which are pure ATP competitors, compounds which are pure substrate competitors, and compounds which are mixed competitors: compete with both ATP and substrate (Levitzki A and Gazit A, Science 1995 Mar 24;267(5205):1782-8). Additional IKK inhibitors include peptide-based substrate competitors endogenously made by the mammalian cell, e.g. PKI (protein kinase inhibitor, Seasholtz AF et al., Proc Natl Acad Sci USA 1995 Feb 28;92(5):1734-8), or proteins inhibiting cdc kinases (Correa-Bordes J and Nurse P, Cell 1995 Dec 15;83(6):1001-9). Additional small peptide based substrate competitive kinase inhibitors and allosteric inhibitors (inhibitory mechanisms independent of ATP or substrate competition) are readily generated by established methods (Hvalby O, et al. Proc Natl Acad Sci USA 1994 May 24;91(11):4761-5; Barja P, et al., Cell Immunol 1994 Jan;153(1):28-38; Villar-Palasi C, Biochim Biophys Acta 1994 Dec 30;1224(3):384-8; Liu WZ, et al., Biochemistry 1994 Aug 23;33(33):10120-6).

TABLE II. Selected Small Molecule IKK Kinase Inhibitors

HA-100 <sup>1</sup>	Iso-H7 <sup>12</sup>	A-3 <sup>18</sup>
Chelerythrine <sup>2</sup>	PKC 19-31	HA1004 <sup>19,20</sup>
Staurosporine <sup>3,4,5</sup>	H-7 <sup>13,3,14</sup>	K-252a <sup>16,5</sup>
Calphostin C <sup>6,7,8,9</sup>	H-89 <sup>15</sup>	KT5823 <sup>16</sup>
K-252b <sup>10</sup>	KT5720 <sup>16</sup>	ML-9 <sup>21</sup>
PKC 19-36 <sup>11</sup>	cAMP-depPKinhib <sup>17</sup>	KT5926 <sup>22</sup>

#### Citations

1. Hagiwara, M., et al. Mol. Pharmacol. 32: 7 (1987)
2. Herbert, J. M., et al. Biochem Biophys Res Com 172: 993 (1990)
3. Schachtele, C., et al. Biochem Biophys Res Com 151: 542 (1988)

4. Tamaoki, T., et al. Biochem Biophys Res Com 135: 397 (1986)
5. Tischler, A. S., et al. J. Neurochemistry 55: 1159 (1990)
6. Bruns, R. F., et al. Biochem Biophys Res Com 176: 288 (1991)
7. Kobayashi, E., et al. Biochem Biophys Res Com 159: 548 (1989)
8. Tamaoki, T., et al. Adv2nd Mass Phosphoprotein Res 24:497(1990)
9. Tamaoki, T., et al. Biotechnology 8: 732 (1990)
10. Yasuzawa, T. J. Antibiotics 39: 1972 (1986)
11. House, C., et al. Science 238: 1726 (1987)
12. Quick, J., et al. Biochem. Biophys. Res. Com. 167: 657 (1992)
13. Bouli, N. M. and Davis, M. Brain Res. 525: 198 (1990)
14. Takahashi, I., et al. J. Pharmacol. Exp. Ther. 255: 1218 (1990)
15. Chijiwa, T., et al. J. Biol. Chem. 265: 5267 (1990)
16. Kase, H., et al. Biochem. Biophys. Res. Com. 142: 436 (1987)
17. Cheng, H. C., et al. J. Biol. Chem. 261: 989 (1986)
18. Inagaki, M., et al. Mol. Pharmacol. 29: 577 (1986)
19. Asano, T. and Hidaka, H. J Pharmacol. Exp Ther 231:141 (1984)
20. Hidaka, H., et al. Biochemistry 23: 5036 (1984)
21. Nagatsu, T., et al. Biochem Biophys Res Com 143:1045 (1987)
22. Nakanishi, S., et al. Mol. Pharmacol. 37: 482 (1990)

20 TABLE III. Selected Peptidyl IKK Kinase Inhibitors

hIkB $\alpha$ , residues 24-39, 32Ala	hIKK- $\alpha$ , $\Delta$ 5-203
hIkB $\alpha$ , residues 29-47, 36Ala	hIKK- $\alpha$ , $\Delta$ 1-178
hIkB $\alpha$ , residues 26-46, 32/36Ala	hIKK- $\alpha$ , $\Delta$ 368-756
hIkB $\beta$ , residues 25-38, 32Ala	hIKK- $\alpha$ , $\Delta$ 460-748
25 hIkB $\beta$ , residues 30-41, 36Ala	hIKK- $\alpha$ , $\Delta$ 1-289
hIkB $\beta$ , residues 26-46, 32/36Ala	hIKK- $\alpha$ , $\Delta$ 12-219
hIkBe, residues 24-40, 32Ala	hIKK- $\alpha$ , $\Delta$ 307-745
hIkBe, residues 31-50, 36Ala	hIKK- $\alpha$ , $\Delta$ 319-644
hIkBe, residues 27-44, 32/36Ala	

30 Accordingly, the invention provides methods for modulating signal transduction

involving I $\kappa$ B in a cell comprising the step of modulating IKK kinase activity, e.g. by contacting the cell with a serine/threonine kinase inhibitor. The cell may reside in culture or in situ, i.e. within the natural host. Preferred inhibitors are orally active in mammalian hosts. For diagnostic uses, the inhibitors or other IKK binding agents are frequently labeled, such as with fluorescent, radioactive, chemiluminescent, or other easily detectable molecules, either conjugated directly to the binding agent or conjugated to a probe specific for the binding agent.

The amino acid sequences of the disclosed IKK- $\alpha$  polypeptides are used to back-translate IKK- $\alpha$  polypeptide-encoding nucleic acids optimized for selected expression systems (Holler et al. (1993) Gene 136, 323-328; Martin et al. (1995) Gene 154, 150-166) or used to generate degenerate oligonucleotide primers and probes for use in the isolation of natural IKK- $\alpha$ -encoding nucleic acid sequences ("GCG" software, Genetics Computer Group, Inc, Madison WI). IKK- $\alpha$ -encoding nucleic acids used in IKK- $\alpha$ -expression vectors and incorporated into recombinant host cells, e.g. for expression and screening, transgenic animals, e.g. for functional studies such as the efficacy of candidate drugs for disease associated with IKK- $\alpha$ -modulated cell function, etc.

The invention also provides nucleic acid hybridization probes and replication / amplification primers having a IKK- $\alpha$  cDNA specific sequence comprising at least 12, preferably at least 24, more preferably at least 36 and most preferably at least contiguous 96 bases of a strand of SEQ ID NO:3, particularly of SEQ ID NO:2, nucleotides 1-1913, and preferably including at least one of bases 1-92, 1811, 1812, 1992, 1995, 2034, 2035, 2039, 2040, 2050, 2055 and 2060, and sufficient to specifically hybridize with a second nucleic acid comprising the complementary strand of SEQ ID NO:3 in the presence of a third nucleic acid comprising (SEQ ID NO:5). Demonstrating specific hybridization generally requires stringent conditions, for example, hybridizing in a buffer comprising 30% formamide in 5 x SSPE (0.18 M NaCl, 0.01 M NaPO<sub>4</sub>, pH7.7, 0.001 M EDTA) buffer at a temperature of 42°C and remaining bound when subject to washing at 42°C with 0.2 x SSPE; preferably hybridizing in a buffer comprising 50% formamide in 5 x SSPE buffer at a temperature of 42°C and remaining bound when subject to washing at 42°C with 0.2 x SSPE buffer at 42°C. IKK- $\alpha$  nucleic acids can also be distinguished using alignment algorithms, such as BLASTX (Altschul *et al.* (1990) Basic Local Alignment Search Tool, J Mol Biol 215, 403-410).

The subject nucleic acids are of synthetic/non-natural sequences and/or are isolated, i.e. unaccompanied by at least some of the material with which it is associated in its natural state, preferably constituting at least about 0.5%, preferably at least about 5% by weight of total nucleic acid present in a given fraction, and usually recombinant, meaning they comprise a non-natural sequence or a natural sequence joined to

5 nucleotide(s) other than that which it is joined to on a natural chromosome. Recombinant nucleic acids comprising the nucleotide sequence of SEQ ID NO:3, or requisite fragments thereof, contain such sequence or fragment at a terminus, immediately flanked by (i.e. contiguous with) a sequence other than that which it is joined to on a natural chromosome, or flanked by a native flanking region fewer than 10 kb, preferably fewer than 2 kb, which

10 is at a terminus or is immediately flanked by a sequence other than that which it is joined to on a natural chromosome. While the nucleic acids are usually RNA or DNA, it is often advantageous to use nucleic acids comprising other bases or nucleotide analogs to provide modified stability, etc.

The subject nucleic acids find a wide variety of applications including use as

15 translatable transcripts, hybridization probes, PCR primers, diagnostic nucleic acids, etc.; use in detecting the presence of IKK- $\alpha$  genes and gene transcripts and in detecting or amplifying nucleic acids encoding additional IKK- $\alpha$  homologs and structural analogs. In diagnosis, IKK- $\alpha$  hybridization probes find use in identifying wild-type and mutant IKK- $\alpha$  alleles in clinical and laboratory samples. Mutant alleles are used to generate allele-specific

20 oligonucleotide (ASO) probes for high-throughput clinical diagnoses. In therapy, therapeutic IKK- $\alpha$  nucleic acids are used to modulate cellular expression or intracellular concentration or availability of active IKK- $\alpha$ .

The invention provides efficient methods of identifying agents, compounds or lead compounds for agents active at the level of a IKK modulatable cellular function.

25 Generally, these screening methods involve assaying for compounds which modulate IKK interaction with a natural IKK binding target, in particular, IKK phosphorylation of I $\kappa$ B-derived substrates, particularly I $\kappa$ B and NIK substrates. A wide variety of assays for binding agents are provided including labeled *in vitro* protein-protein binding assays, immunoassays, cell based assays, etc. The methods are amenable to automated, cost-

30 effective high throughput screening of chemical libraries for lead compounds. Identified reagents find use in the pharmaceutical industries for animal and human trials; for example,

the reagents may be derivatized and rescreened in *in vitro* and *in vivo* assays to optimize activity and minimize toxicity for pharmaceutical development.

*In vitro* binding assays employ a mixture of components including an IKK polypeptide, which may be part of a fusion product with another peptide or polypeptide, e.g. a tag for detection or anchoring, etc. The assay mixtures comprise a natural intracellular IKK binding target. In a particular embodiment, the binding target is a substrate comprising IkB serines 32 and/or 36. Such substrates comprise a IkB $\alpha$ ,  $\beta$  or  $\epsilon$  peptide including the serine 32 and/or 36 residue and at least 5, preferably at least 10, and more preferably at least 20 naturally occurring immediately flanking residues on each side (e.g. for serine 36 peptides, residues 26-46, 22-42, or 12-32 or 151-171 for IkB $\alpha$ ,  $\beta$  or  $\epsilon$ -derived substrates, respectively). While native full-length binding targets may be used, it is frequently preferred to use portions (e.g. peptides) thereof so long as the portion provides binding affinity and avidity to the subject IKK polypeptide conveniently measurable in the assay. The assay mixture also comprises a candidate pharmacological agent. Candidate agents encompass numerous chemical classes, though typically they are organic compounds; preferably small organic compounds and are obtained from a wide variety of sources including libraries of synthetic or natural compounds. A variety of other reagents may also be included in the mixture. These include reagents like ATP or ATP analogs (for kinase assays), salts, buffers, neutral proteins, e.g. albumin, detergents, protease inhibitors, nuclease inhibitors, antimicrobial agents, etc. may be used.

The resultant mixture is incubated under conditions whereby, but for the presence of the candidate pharmacological agent, the IKK polypeptide specifically binds the cellular binding target, portion or analog with a reference binding affinity. The mixture components can be added in any order that provides for the requisite bindings and incubations may be performed at any temperature which facilitates optimal binding. Incubation periods are likewise selected for optimal binding but also minimized to facilitate rapid, high-throughput screening.

After incubation, the agent-biased binding between the IKK polypeptide and one or more binding targets is detected by any convenient way. For IKK kinase assays, 'binding' is generally detected by a change in the phosphorylation of a IKK- $\alpha$  substrate. In this embodiment, kinase activity may be quantified by the transfer to the substrate of a labeled phosphate, where the label may provide for direct detection as radioactivity, luminescence,

optical or electron density, etc. or indirect detection such as an epitope tag, etc. A variety of methods may be used to detect the label depending on the nature of the label and other assay components, e.g. through optical or electron density, radiative emissions, nonradiative energy transfers, etc. or indirectly detected with antibody conjugates, etc.

5 A difference in the binding affinity of the IKK polypeptide to the target in the absence of the agent as compared with the binding affinity in the presence of the agent indicates that the agent modulates the binding of the IKK polypeptide to the IKK binding target. Analogously, in the cell-based assay also described below, a difference in IKK- $\alpha$ -dependent transcriptional activation in the presence and absence of an agent indicates the agent modulates IKK function. A difference, as used herein, is statistically significant and  
10 preferably represents at least a 50%, more preferably at least a 90% difference.

The following experimental section and examples are offered by way of illustration and not by way of limitation.

## EXPERIMENTAL

### 15 Identification of IKK- $\alpha$

To investigate the mechanism of NIK-mediated NF- $\kappa$ B activation, we identified proteins that associate directly with NIK by yeast two-hybrid protein interaction cloning (Fields and Song, 1989). An expression vector was generated that encodes NIK fused to the DNA-binding domain of the yeast transcription factor GAL4. This vector was used as bait in  
20 a two-hybrid screen of a human B cell cDNA library. From approximately six million transformants, eight positive clones were obtained, as determined by activation of *his* and *lacZ* reporter genes. Of these clones, three encoded a member of the TRAF family, TRAF3 (Hu et al., 1994; Cheng et al., 1995; Mosialos et al., 1995; Sato et al., 1995) and one encoded a novel protein we call IKK- $\alpha$ . Retransformation into yeast cells verified the interaction between  
25 NIK and IKK- $\alpha$ . A full-length human IKK- $\alpha$  clone was isolated by screening a Jurkat cDNA library with a probe generated from the 5'-end of the IKK- $\alpha$  two-hybrid clone. IKK- $\alpha$  comprises an N-terminal serine-threonine kinase catalytic domain, a C-terminal helix-loop-helix domain and a leucine zipper-like amphipathic  $\alpha$ -helix juxtaposed in between the helix-loop-helix and kinase domain.

### 30 Interaction of IKK- $\alpha$ and NIK in Human Cells

The interaction of IKK- $\alpha$  with NIK was confirmed in mammalian cell

coimmunoprecipitation assays. Human IKK- $\alpha$  containing an N-terminal Flag epitope tag was transiently coexpressed in 293 human embryonic kidney cells with Myc epitope-tagged NIK or HA epitope-tagged TRAF proteins. Cell lysates were immunoprecipitated using a monoclonal antibody against the Flag epitope, and coprecipitating NIK or TRAF proteins were detected by immunoblot analysis with an anti-Myc or anti-HA monoclonal antibodies.

5 In this assay, IKK- $\alpha$  was able to coprecipitate NIK confirming the interaction between both proteins as detected for IKK- $\alpha$  by yeast two-hybrid analysis. Also, a deletion mutant IKK- $\alpha$  protein lacking most of the N-terminal kinase domain (IKK- $\alpha_{(307-745)}$ ) was able to associate with NIK, indicating that the  $\alpha$ -helical C-terminal half of IKK- $\alpha$  mediates the interaction with NIK. In contrast to NIK, IKK- $\alpha$  failed to associate with either TRAF2 or TRAF3. However, 10 when NIK was coexpressed with IKK- $\alpha$  and TRAF2, strong coprecipitation of TRAF2 with IKK- $\alpha$  was detected, indicating the formation of a ternary complex between IKK- $\alpha$ , NIK and TRAF2.

#### Effect of IKK- $\alpha$ and IKK- $\alpha$ Mutants on NF- $\kappa$ B Activation

To investigate a possible role for IKK- $\alpha$  in NF- $\kappa$ B activation, we examined if transient 15 overexpression of IKK- $\alpha$  might activate an NF- $\kappa$ B-dependent reporter gene. An E-selectin-luciferase reporter construct (Schindler and Baichwal, 1994) and a IKK- $\alpha$  expression vector were cotransfected into HeLa cells. IKK- $\alpha$  expression activated the reporter gene in a dose-dependent manner, with a maximal induction of luciferase activity of about 6 to 7-fold compared to vector control. Similar results were obtained in 293 cells, where IKK- $\alpha$  20 overexpression induced reporter gene activity approximately 4-fold. TNF treatment did not stimulate the weak NF- $\kappa$ B-inducing activity of overexpressed IKK- $\alpha$  in reporter gene assays. Thus, IKK- $\alpha$  induces NF- $\kappa$ B activation when overexpressed.

We next determined the effect of overexpression of kinase-inactive IKK- $\alpha_{(307-745)}$  that still associates with NIK on signal-induced NF- $\kappa$ B activation in reporter gene assays in 25 293 cells. Overexpression of IKK- $\alpha_{(307-745)}$  blocked TNF- and IL-1-induced reporter gene activation similar to overexpression of NIK<sub>(624-947)</sub>. IKK- $\alpha_{(307-745)}$  was also found to inhibited NF- $\kappa$ B-dependent reporter gene activity elicited by overexpression of TRAF2, TRAF6 and NIK. Taken together these results demonstrate that a catalytically inactive IKK- $\alpha$  mutant is a dominant-negative inhibitor of TNF-, IL-1, TRAF- and NIK-induced NF- $\kappa$ B 30 activation. This indicates that IKK- $\alpha$  functions as a common mediator of NF- $\kappa$ B activation by TNF and IL-1 downstream of NIK.

Parenthetical References

- Ansieau, S., et al. (1996). *Proc. Natl. Acad. Sci. USA* **93**, 14053-14058.
- Baeuerle, P. A., and Henkel, T. (1994). *Annu. Rev. Immunol.* **12**, 141-179.
- Beg, A. A., et al. (1993). *Mol. Cell. Biol.* **13**, 3301-3310.
- Cao, Z., Henzel, W. J., and Gao, X. (1996a). *Science* **271**, 1128-1131.
- 5 Cao, Z., et al. (1996b). *Nature* **383**, 443-446.
- Chen, Z., et al. (1995). *Genes Dev.* **9**, 1586-1597.
- Cheng, G., et al. (1995). *Science* **267**, 1494-1498.
- Connelly, M. A., and Marcu, K. B. (1995). *Cell. Mol. Biol. Res.* **41**, 537-549.
- Dinarello, C. A. (1996). Biologic basis for interleukin-1 in disease. *Blood* **87**, 2095-2147.
- 10 Fields, S., and Song, O.-k. (1989). *Nature* **340**, 245-246.
- Finco, T. S., and Baldwin, A. S. (1995). *Immunity* **3**, 263-272.
- Gedrich, R. W., et al. (1996). *J. Biol. Chem.* **271**, 12852-12858.
- Hill, C. S., and Treisman, R. (1995). *Cell* **80**, 199-211.
- Hsu, H., Shu, H.-B., Pan, M.-P., and Goeddel, D. V. (1996). *Cell* **84**, 299-308.
- 15 Hu, H. M., et al. (1994). *J. Biol. Chem.* **269**, 30069-30072.
- Lee, S. Y., et al. (1996). *Proc. Natl. Acad. Sci. USA* **93**, 9699-9703.
- Lenardo, M., and Baltimore, D. (1989). *Cell* **58**, 227-229.
- Malinin, N. L., et al. (1997). *Nature* **385**, 540-544.
- Mock et al. (1995). *Genomics* **27**, 348-351.
- 20 Mosialos, G., et al. (1995). *Cell* **80**, 389-399.
- Nakano, H., et al. (1996). *J. Biol. Chem.* **271**, 14661-14664.
- Osborn, L., Kunkel, S., and Nabel, G. J. (1989). *Proc. Natl. Acad. Sci. USA* **86**, 2336-2340.
- Rothe, M., Sarma, V., Dixit, V. M., and Goeddel, D. V. (1995). *Science* **269**, 1424-1427.
- Rothe, M., Wong, S. C., Henzel, W. J., and Goeddel, D. V. (1994). *Cell* **78**, 681-692.
- 25 Sato, T., Irie, S., and Reed, J. C. (1995). *FEBS Lett.* **358**, 113-118.
- Schindler, U., and Baichwal, V. R. (1994). *Mol. Cell. Biol.* **14**, 5820-5831.
- Smith, C. A., Farrah, T., and Goodwin, R. G. (1994). *Cell* **76**, 959-962.
- Song, H. Y., and Donner, D. B. (1995). *Biochem. J.* **309**, 825-829.
- Thanos, D., and Maniatis, T. (1995). *Cell* **80**, 529-532.
- 30 Verma, I. M., et al. (1995). *Genes Dev.* **9**, 2723-2735.

## EXAMPLES

1. Protocol for at IKK- $\alpha$  - I $\kappa$ B $\alpha$  phosphorylation assay.
- A. Reagents:
  - NeutrAvidin: 20  $\mu$ g/ml in PBS.
  - kinase:  $10^{-8}$  -  $10^{-5}$  M IKK- $\alpha$  (SEQ ID NO:4) at 20  $\mu$ g/ml in PBS.
  - 5 - substrate:  $10^{-7}$  -  $10^{-4}$  M biotinylated substrate (21 residue peptide consisting of residues 26-46 of human I $\kappa$ B $\alpha$ ) at 40  $\mu$ g/ml in PBS.
  - Blocking buffer: 5% BSA, 0.5% Tween 20 in PBS; 1 hour at room temperature.
  - Assay Buffer: 100 mM KCl, 10 mM MgCl<sub>2</sub>, 1 mM MnCl<sub>2</sub>, 20 mM HEPES pH 7.4, 0.25 mM EDTA, 1% glycerol, 0.5% NP-40, 50 mM BME, 1 mg/ml BSA, cocktail of protease  
10 inhibitors.
  - [<sup>32</sup>P]γ-ATP 10x stock:  $2 \times 10^{-5}$  M cold ATP with 100  $\mu$ Ci [<sup>32</sup>P]γ-ATP. Place in the 4°C microfridge during screening.
  - Protease inhibitor cocktail (1000X): 10 mg Trypsin Inhibitor (BMB # 109894), 10 mg Aprotinin (BMB # 236624), 25 mg Benzamidine (Sigma # B-6506), 25 mg Leupeptin  
15 (BMB # 1017128), 10 mg APMSF (BMB # 917575), and 2mM NaVO<sub>3</sub> (Sigma # S-6508) in 10 ml of PBS.
- B. Preparation of assay plates:
  - Coat with 120  $\mu$ l of stock N Avidin per well overnight at 4°C.
  - Wash 2 times with 200  $\mu$ l PBS.
  - 20 - Block with 150  $\mu$ l of blocking buffer.
  - Wash 2 times with 200  $\mu$ l PBS.
- C. Assay:
  - Add 40  $\mu$ l assay buffer/well.
  - Add 40  $\mu$ l biotinylated substrate (2-200 pmoles/40  $\mu$ l in assay buffer)
  - 25 - Add 40  $\mu$ l kinase (0.1-10 pmoles/40  $\mu$ l in assay buffer)
  - Add 10  $\mu$ l compound or extract.
  - Add 10  $\mu$ l [<sup>32</sup>P]γ-ATP 10x stock.
  - Shake at 25°C for 15 minutes.
  - Incubate additional 45 minutes at 25°C.
  - 30 - Stop the reaction by washing 4 times with 200  $\mu$ l PBS.
  - Add 150  $\mu$ l scintillation cocktail.

- Count in Topcount.
- D. Controls for all assays (located on each plate):
  - a. Non-specific binding
  - b. cold ATP at 80% inhibition.
- 5 2. Protocol for high throughput IKK- $\alpha$ -NIK binding assay.
- A. Reagents:
  - Neutralite Avidin: 20  $\mu$ g/ml in PBS.
  - Blocking buffer: 5% BSA, 0.5% Tween 20 in PBS; 1 hour at room temperature.
  - Assay Buffer: 100 mM KCl, 20 mM HEPES pH 7.6, 1 mM  $MgCl_2$ , 1% glycerol,
  - 10 0.5% NP-40, 50 mM  $\beta$ -mercaptoethanol, 1 mg/ml BSA, cocktail of protease inhibitors.
  - $^{33}P$  IKK- $\alpha$  polypeptide 10x stock:  $10^{-3}$  -  $10^{-6}$  M "cold" IKK- $\alpha$  supplemented with 200,000-250,000 cpm of labeled IKK- $\alpha$  (Beckman counter). Place in the 4°C microfridge during screening.
  - Protease inhibitor cocktail (1000X): 10 mg Trypsin Inhibitor (BMB # 109894), 10
  - 15 mg Aprotinin (BMB # 236624), 25 mg Benzamidine (Sigma # B-6506), 25 mg Leupeptin (BMB # 1017128), 10 mg APMSF (BMB # 917575), and 2mM  $NaVO_3$  (Sigma # S-6508) in 10 ml of PBS.
  - NIK:  $10^{-7}$  -  $10^{-5}$  M biotinylated NIK in PBS.
- B. Preparation of assay plates:
  - 20 - Coat with 120  $\mu$ l of stock N-Avidin per well overnight at 4°C.
  - Wash 2 times with 200  $\mu$ l PBS.
  - Block with 150  $\mu$ l of blocking buffer.
  - Wash 2 times with 200  $\mu$ l PBS.
- C. Assay:
  - 25 - Add 40  $\mu$ l assay buffer/well.
  - Add 10  $\mu$ l compound or extract.
  - Add 10  $\mu$ l  $^{33}P$ -IKK- $\alpha$  (20-25,000 cpm/0.1-10 pmoles/well =  $10^{-9}$  -  $10^{-7}$  M final conc).
  - Shake at 25°C for 15 minutes.
  - Incubate additional 45 minutes at 25°C.
  - 30 - Add 40  $\mu$ M biotinylated NIK (0.1-10 pmoles/40  $\mu$ l in assay buffer)
  - Incubate 1 hour at room temperature.

- Stop the reaction by washing 4 times with 200  $\mu$ M PBS.
- Add 150  $\mu$ M scintillation cocktail.
- Count in Topcount.

D. Controls for all assays (located on each plate):

- a. Non-specific binding
- b. Soluble (non-biotinylated NIK) at 80% inhibition.

5

10

All publications and patent applications cited in this specification are herein incorporated by reference as if each individual publication or patent application were specifically and individually indicated to be incorporated by reference. Although the foregoing invention has been described in some detail by way of illustration and example for purposes of clarity of understanding, it will be readily apparent to those of ordinary skill in the art in light of the teachings of this invention that certain changes and modifications may be made thereto without departing from the spirit or scope of the appended claims.

## WHAT IS CLAIMED IS:

1. An isolated polypeptide comprising SEQ ID NO:4, or at least a 10 residue domain thereof comprising at least one of Cys30, Leu604, Thr679, Ser680, Pro684, Thr686 and Ser678.
- 5 2. An isolated polypeptide according to claim 1, wherein said polypeptide has an activity selected from at least one of: a kinase or kinase inhibitory activity, a NIK-binding or binding inhibitory activity, an IκB-binding or binding inhibitory activity and an NFκB activating or inhibitory activity.
- 10 3. An isolated or recombinant first nucleic acid comprising a strand of SEQ ID NO:3, or a portion thereof having at least 24 contiguous bases of SEQ ID NO:3 and including at least one of bases 1-92, 1811, 1812, 1992, 1995, 2034, 2035, 2039, 2040, 2050, 2055 and 2060, sufficient to specifically hybridize with a second nucleic acid comprising the complementary strand of SEQ ID NO:3 in the presence of a third nucleic acid comprising  
15 (SEQ ID NO:5).
4. A recombinant nucleic acid encoding a polypeptide according to claim 1.
5. A cell comprising a nucleic acid according to claim 4.
- 20 6. A method of making an isolated polypeptide according to claim 1, said method comprising steps: introducing a nucleic acid according to claim 4 into a host cell or cellular extract, incubating said host cell or extract under conditions whereby said nucleic acid is expressed as a transcript and said transcript is expressed as a translation product comprising  
25 said polypeptide, and isolating said translation product.
7. A method of screening for an agent which modulates the interaction of an IKK polypeptide to a binding target, said method comprising the steps of:  
incubating a mixture comprising:  
30 an isolated polypeptide according to claim 1,  
a binding target of said polypeptide, and

a candidate agent;

under conditions whereby, but for the presence of said agent, said polypeptide specifically binds said binding target at a reference affinity;

detecting the binding affinity of said polypeptide to said binding target to determine an agent-biased affinity, wherein a difference between the agent-biased affinity and the reference affinity indicates that said agent modulates the binding of said polypeptide to said binding target.

8. A method according to claim 7, wherein said binding target is a natural intracellular substrate and said reference and agent-biased binding affinity is detected as phosphorylation of said substrate.

9. A method of screening for an agent which modulates the interaction of an IKK polypeptide to a binding target, said method comprising the steps of:

incubating a mixture comprising: an isolated polypeptide comprising SEQ ID NO: 2 or 4, or a deletion mutant thereof retaining I $\kappa$ B kinase activity, an I $\kappa$ B polypeptide comprising at least a six residue domain of a natural I $\kappa$ B comprising at least one of Ser32 and Ser 36, and a candidate agent;

under conditions whereby, but for the presence of said agent, said polypeptide specifically phosphorylates said I $\kappa$ B polypeptide at at least one of said Ser32 and Ser36 at a reference activity;

detecting the polypeptide-induced phosphorylation of said I $\kappa$ B polypeptide at at least one of said Ser32 and Ser36 to determine an agent-biased activity, wherein a difference between the agent-biased activity and the reference activity indicates that said agent modulates the ability of said polypeptide to specifically phosphorylate a I $\kappa$ B polypeptide.

10. A method for modulating signal transduction involving I $\kappa$ B in a cell, said method comprising the step of modulating IKK- $\alpha$  (SEQ ID NO:4) kinase activity.

11. The method of claim 10, wherein said modulating step comprises contacting the cell with a serine/threonine kinase inhibitor.

## SEQUENCE LISTING

## (1) GENERAL INFORMATION:

- 5 (i) APPLICANT: Rothe, Mike  
CaO, Zhaoan  
Régnier, Catherine
- (ii) TITLE OF INVENTION: IKK- $\alpha$  Proteins, Nucleic Acids and Methods
- 10 (iii) NUMBER OF SEQUENCES: 5
- (iv) CORRESPONDENCE ADDRESS:  
(A) ADDRESSEE: SCIENCE & TECHNOLOGY LAW GROUP  
(B) STREET: 268 BUSH STREET, SUITE 3200  
15 (C) CITY: SAN FRANCISCO  
(D) STATE: CALIFORNIA  
(E) COUNTRY: USA  
(F) ZIP: 94104
- 20 (v) COMPUTER READABLE FORM:  
(A) MEDIUM TYPE: Floppy disk  
(B) COMPUTER: IBM PC compatible  
(C) OPERATING SYSTEM: PC-DOS/MS-DOS  
(D) SOFTWARE: PatentIn Release #1.0, Version #1.30
- 25 (vi) CURRENT APPLICATION DATA:  
(A) APPLICATION NUMBER:  
(B) FILING DATE:  
(C) CLASSIFICATION:
- 30 (viii) ATTORNEY/AGENT INFORMATION:  
(A) NAME: OSMAN, RICHARD A  
(B) REGISTRATION NUMBER: 36,627  
(C) REFERENCE/DOCKET NUMBER: T97-006-1
- 35 (ix) TELECOMMUNICATION INFORMATION:  
(A) TELEPHONE: (415) 343-4341  
(B) TELEFAX: (415) 343-4342
- 40 (2) INFORMATION FOR SEQ ID NO:1:  
(i) SEQUENCE CHARACTERISTICS:  
(A) LENGTH: 2268 base pairs

(B) TYPE: nucleic acid  
 (C) STRANDEDNESS: double  
 (D) TOPOLOGY: linear

(ii) MOLECULE TYPE: cDNA

5

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:1:

	ATGAGCTGGT	CACCTCCCT	GACAACGCAG	ACATGTGGGG	CCTGGGAAAT	GAAAGAGCGC	60
	CTTGGGACAG	GGGGATTGG	AAATGTCATC	CGATGGCACA	ATCAGGAAAC	AGGTGAGCAG	120
	ATTGCCATCA	AGCAGTCCG	GCAGGAGCTC	AGCCCCCGGA	ACCGAGAGCG	GTGTGCGCTG	180
10	GAGATCCAGA	TCATGAGAAG	GCTGACCCAC	CCCAATGTGG	TGGCTGCCCG	AGATGTCCCT	240
	GAGGGGATGC	AGAACTTGGC	GOCCAAATGAC	CTGCCCTGTC	TGGCCATGGA	GTACTGCCAA	300
	GGAGGAGATC	TCCGGAAGTA	CCTGAACCCAG	TTTGAGAACT	GCTGTGTCT	CGCGGAAGST	360
	GCCATCCTCA	CCTGTCTGAG	TGACATTGCC	TCTGCGCTTA	GATACCTTCA	TGAAAACAGA	420
	ATCATCCATC	GGGATCTAAA	GCCAGAAAAC	ATCGTCTCTG	AGCAGGAGAG	AGTACGGTTA	480
15	ATACACAAAA	TTATTGAOCT	AGGATATGCC	AAGGAGCTGG	ATCAGGGCAG	TCITTGCACA	540
	TCATTCTGTG	GGACCCCTGA	GTACCTGGCC	CCAGAGCTAC	TGGAGCAGCA	GAAGTACACA	600
	GTGACCGTGG	ACTACTGGAG	CTTCGGCACC	CTGGCCTTTG	AGTGATCAC	GGGCTTCCGG	660
	CCCTTCTCTC	CCAACTGGCA	GCCCGTGCAG	TGGCAITCAA	AAGTGCGGCA	GAAGAGTGAG	720
	GTGGACATTG	TTGTAGCGA	AGACTTGAAT	GGAACGGTGA	AGTTTTCAAG	CTCTTTACCC	780
20	TACCCCAATA	ATCTTAAACAG	TGTCTGGCT	GAGCGACTGG	AGAAGTGGCT	GCACTGATG	840
	CTGATGTGGC	ACCCCGGACA	GAGGGGCACG	GATCCACGTT	ATGGGCCCAA	TGGCTGCTTC	900
	AAGGCCCTGG	ATGACATCTT	AAACTTAAAG	CTGTTTCATA	TCTTGAACAT	GGTCACGGCG	960
	ACCATCCACA	CCTACCTGTT	GACAGAGGAT	GAGAGTCTCG	AGAGCTTGAA	GGCCAGAATC	1020
	CAACAGGACA	CGGGCATCCC	AGAGGAGGAC	CAGGAGCTGC	TGCAGGAAGC	GGGCTTGGCG	1080
25	TTGATCCCCG	ATAAGCGCTG	CACCTCAGTT	ATTTCAGACG	GCAAGTTAAA	TGAGGGCCAC	1140
	ACATTTGACA	TGGATCTGTT	TTTTCTCTTT	GACACAGATA	AAATCACTTA	TGAGACTCAG	1200
	ATCTCCCCAC	GGCCCCRACC	TGAAAGTGTC	AGCTGTATCC	TTCAAGAGCC	CAAGAGGAAT	1260
	CTGCGCTTCT	TCCAGCTGAG	GAAGGTGTGG	GGCCAGGTCT	GGCACAGCAT	CCAGACCCCTG	1320
	AAGGAAGATT	GCAACCGGCT	GCAGCAGGGA	CAGCGAGCCG	CCATGATGAA	TCTCTCCGGA	1380
30	AACAACAGCT	GCCCTTCCAA	AATGAAGAAT	TCCATGGCTT	CCATGTCTCA	GCAGCTCAAG	1440
	GCCAGTTTGG	ATTCTTCTAA	AACCAGCATC	CAGATTGACC	TGGAGAAGTA	CAGCGAGCAA	1500
	ACCGAGTTTG	GGATCAGATC	AGATAAACTG	CTGCTGGCCT	GGAGGGAAAT	GGAGCAGGCT	1560
	GTGAGAGCTCT	GTTGGGCGGA	GAAOAGAGTG	AAACTCCTGG	TAGAACGGAT	GATGGCTCTG	1620
	CAGACCGACA	TTTGTGACTT	ACAGAGGAGC	CCCATTGGGC	GGAAGCAGGG	GGGAACGCTG	1680
35	GACGACCTAG	AGGAGCAGCG	AAGGGAGCTG	TACAGGAGAC	TAGGGGAAAA	ACCTCGAGAC	1740
	CAGCGAACTG	AGGGTGACAG	TCAGGAAATG	GTACGGCTGC	TGCTTCAGGC	AAATTCAGAG	1800
	TTCCGAGAAGA	AAGTGCGAGT	GATCTATACG	CAGCTCAGTA	AAACTGTGGT	TTGCAAGCAG	1860
	AAGGCGCTGG	AACTGTGTCC	CAAGGTGGAA	GAGGTGGTGA	GCTTAATGAA	TGAGGATGAG	1920
	AAGACTGTGT	TCCGGCTGCA	GGAGAAGCGG	CAGAAGGAGC	TCTGGAATCT	CCTGAAGATT	1980
40	GCTTGTATGA	AGGTCGGTGG	TCCTGTCTAGT	GGAAGCCCGG	ATAGCATGAA	TGCGCTCTGA	2040
	CTTAGCCAGC	TCTGGGCGCT	GATGTCTCAG	CCCTCCACGG	CCTCCAACAG	CTTACCTGAG	2100
	CCAGCCACGA	AGAGTGAGAA	ACTGGTGGCT	GAAGCACATA	ACCTCTGCAC	CCTGCTAGAA	2160
	AATGCCATAC	AGGACATCTG	GAGGGAACAA	GACCAGAGTT	TCACGGCCCT	AGACTGGAGC	2220
	TGGTTACAGA	CGGAAGAAGA	AGAGCACAGC	TGCCTGGAGC	AGGCCTCA		2268

## (2) INFORMATION FOR SEQ ID NO:2:

## (i) SEQUENCE CHARACTERISTICS:

(A) LENGTH: 756 amino acids

(B) TYPE: amino acid

(C) STRANDEDNESS: single

(D) TOPOLOGY: linear

## (ii) MOLECULE TYPE: peptide

## (xi) SEQUENCE DESCRIPTION: SEQ ID NO:2:

10 Met Ser Trp Ser Pro Ser Leu Thr Thr Gln Thr Cys Gly Ala Trp Glu  
 1 5 10 15  
 Met Lys Glu Arg Leu Gly Thr Gly Gly Phe Gly Asn Val Ile Arg Trp  
 20 25 30  
 15 His Asn Gln Glu Thr Gly Glu Gln Ile Ala Ile Lys Gln Cys Arg Gln  
 35 40 45  
 Glu Leu Ser Pro Arg Asn Arg Glu Arg Trp Cys Leu Glu Ile Gln Ile  
 50 55 60  
 Met Arg Arg Leu Thr His Pro Asn Val Val Ala Ala Arg Asp Val Pro  
 20 65 70 75 80  
 Glu Gly Met Gln Asn Leu Ala Pro Asn Asp Leu Pro Leu Leu Ala Met  
 85 90 95  
 Glu Tyr Cys Gln Gly Gly Asp Leu Arg Lys Tyr Leu Asn Gln Phe Glu  
 100 105 110  
 25 Asn Cys Cys Gly Leu Arg Glu Gly Ala Ile Leu Thr Leu Leu Ser Asp  
 115 120 125  
 Ile Ala Ser Ala Leu Arg Tyr Leu His Glu Asn Arg Ile Ile His Arg  
 130 135 140  
 Asp Leu Lys Pro Glu Asn Ile Val Leu Gln Gln Gly Glu Gln Arg Leu  
 30 145 150 155 160  
 Ile His Lys Ile Ile Asp Leu Gly Tyr Ala Lys Glu Leu Asp Gln Gly  
 165 170 175  
 Ser Leu Cys Thr Ser Phe Val Gly Thr Leu Gln Tyr Leu Ala Pro Glu  
 180 185 190  
 35 Leu Leu Glu Gln Gln Lys Tyr Thr Val Thr Val Asp Tyr Trp Ser Phe  
 195 200 205  
 Gly Thr Leu Ala Phe Glu Cys Ile Thr Gly Phe Arg Pro Phe Leu Pro  
 210 215 220  
 Asn Trp Gln Pro Val Gln Trp His Ser Lys Val Arg Gln Lys Ser Glu  
 40 225 230 235 240  
 Val Asp Ile Val Val Ser Glu Asp Leu Asn Gly Thr Val Lys Phe Ser  
 245 250 255  
 Ser Ser Leu Pro Tyr Pro Asn Asn Leu Asn Ser Val Leu Ala Glu Arg  
 260 265 270

	Leu	Glu	Lys	Trp	Leu	Gln	Leu	Met	Leu	Met	Trp	His	Pro	Arg	Gln	Arg
	275							280					285			
	Gly	Thr	Asp	Pro	Thr	Tyr	Gly	Pro	Asn	Gly	Cys	Phe	Lys	Ala	Leu	Asp
	290						295				300					
5	Asp	Ile	Leu	Asn	Leu	Lys	Leu	Val	His	Ile	Leu	Asn	Met	Val	Thr	Gly
	305				310						315				320	
	Thr	Ile	His	Thr	Tyr	Pro	Val	Thr	Glu	Asp	Glu	Ser	Leu	Gln	Ser	Leu
				325						330					335	
	Lys	Ala	Arg	Ile	Gln	Gln	Asp	Thr	Gly	Ile	Pro	Glu	Glu	Asp	Gln	Glu
				340					345					350		
10	Leu	Leu	Gln	Glu	Ala	Gly	Leu	Ala	Leu	Ile	Pro	Asp	Lys	Pro	Ala	Thr
		355					360						365			
	Gln	Cys	Ile	Ser	Asp	Gly	Lys	Leu	Asn	Glu	Gly	His	Thr	Leu	Asp	Met
	370					375						380				
	Asp	Leu	Val	Phe	Leu	Phe	Asp	Asn	Ser	Lys	Ile	Thr	Tyr	Glu	Thr	Gln
15	385				390										400	
	Ile	Ser	Pro	Arg	Pro	Gln	Pro	Glu	Ser	Val	Ser	Cys	Ile	Leu	Gln	Glu
				405						410					415	
	Pro	Lys	Arg	Asn	Leu	Ala	Phe	Phe	Gln	Leu	Arg	Lys	Val	Trp	Gly	Gln
				420					425					430		
20	Val	Trp	His	Ser	Ile	Gln	Thr	Leu	Lys	Glu	Asp	Cys	Asn	Arg	Leu	Gln
		435					440						445			
	Gln	Gly	Gln	Arg	Ala	Ala	Met	Met	Asn	Leu	Leu	Arg	Asn	Asn	Ser	Cys
	450					455					460					
25	Leu	Ser	Lys	Met	Lys	Asn	Ser	Met	Ala	Ser	Met	Ser	Gln	Gln	Leu	Lys
	465				470						475				480	
	Ala	Lys	Leu	Asp	Phe	Phe	Lys	Thr	Ser	Ile	Gln	Ile	Asp	Leu	Glu	Lys
				485						490					495	
	Tyr	Ser	Glu	Gln	Thr	Glu	Phe	Gly	Ile	Thr	Ser	Asp	Lys	Leu	Leu	Leu
		500						505						510		
30	Ala	Trp	Arg	Glu	Met	Glu	Gln	Ala	Val	Glu	Leu	Cys	Gly	Arg	Glu	Asn
		515					520						525			
	Glu	Val	Lys	Leu	Leu	Val	Glu	Arg	Met	Met	Ala	Leu	Gln	Thr	Asp	Ile
	530					535						540				
	Val	Asp	Leu	Gln	Arg	Ser	Pro	Met	Gly	Arg	Lys	Gln	Gly	Gly	Thr	Leu
35	545				550						555				560	
	Asp	Asp	Leu	Glu	Glu	Gln	Ala	Arg	Glu	Leu	Tyr	Arg	Arg	Leu	Arg	Glu
				565						570					575	
	Lys	Pro	Arg	Asp	Gln	Arg	Thr	Glu	Gly	Asp	Ser	Gln	Glu	Met	Val	Arg
				580					585					590		
40	Leu	Leu	Leu	Gln	Ala	Ile	Gln	Ser	Phe	Glu	Lys	Lys	Val	Arg	Val	Ile
		595					600						605			
	Tyr	Thr	Gln	Leu	Ser	Lys	Thr	Val	Val	Cys	Lys	Gln	Lys	Ala	Leu	Glu
	610						615					620				
	Leu	Leu	Pro	Lys	Val	Glu	Glu	Val	Val	Ser	Leu	Met	Asn	Glu	Asp	Glu

	625		630		635		640
	Lys Thr Val Val Arg Leu Gln Glu Lys Arg Gln Lys Glu Leu Trp Asn						
		645		650		655	
	Leu Leu Lys Ile Ala Cys Ser Lys Val Arg Gly Pro Val Ser Gly Ser						
		660		665		670	
5	Pro Asp Ser Met Asn Ala Ser Arg Leu Ser Gln Pro Gly Gln Leu Met						
		675		680		685	
	Ser Gln Pro Ser Thr Ala Ser Asn Ser Leu Pro Glu Pro Ala Lys Lys						
		690		695		700	
	Ser Glu Glu Leu Val Ala Glu Ala His Asn Leu Cys Thr Leu Leu Glu						
10	705		710		715		720
	Asn Ala Ile Gln Asp Thr Val Arg Glu Gln Asp Gln Ser Phe Thr Ala						
		725		730		735	
	Leu Asp Trp Ser Trp Leu Gln Thr Glu Glu Glu Glu His Ser Cys Leu						
		740		745		750	
15	Glu Gln Ala Ser						
	755						

## (2) INFORMATION FOR SEQ ID NO:3:

- 20 (i) SEQUENCE CHARACTERISTICS:
- (A) LENGTH: 2238 base pairs
- (B) TYPE: nucleic acid
- (C) STRANDEDNESS: double
- (D) TOPOLOGY: linear

25

## (ii) MOLECULE TYPE: cDNA

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:3:

	ATGGAGCGGC	CCCCGGGCT	GCGGCGGGC	GCGGCGGGC	CCTGGGAGAT	GCGGGAGCGG	60
30	CTGGGCACGG	GCGGCTGGG	GAACTCTGT	CTGTACCAGC	ATCGGGAAC	TGATCTCAAA	120
	ATAGCAATTA	AGTCTTGTG	CCTAGAGCTA	AGTACCAAAA	ACAGAGAACC	ATGGTGCCAT	180
	GAAATCCAGA	TTATGAAGAA	GTGTAAACAT	GCCAATGTTG	TAAAGGCGCT	TGATGTTCT	240
	GAAGAATTGA	ATATTTTGT	TCATGATGTG	CCTCTTCTAG	CAATGGAATA	CTGTTCTGGA	300
	GGAGATCTCC	GAAAGCTGCT	CAACAAACCA	GAAAATTGTT	GTGGACTTAA	AGAAAGCCAG	360
35	ATACITTTCT	TACTAAGTGA	TATAGGGTCT	GGGATTCGAT	ATTTCATGTA	AAACAAAATT	420
	ATACATCGAG	ATCTAAACCC	TGAAAACATA	GTTCTTCAGG	ATGTTGGTGG	AAAGATAATA	480
	CATAAAATAA	TTGATCTGGG	ATATGCCAAA	GATGTTGATC	AAGGAAGTCT	GTGTACTACT	540
	TTTGTGGGAA	CACGTGAGTA	TCGTGCCCCA	GAGCTCTTTG	AGAATAAGCC	TTACACAGCC	600
	ACTGTGATTA	ATTGGAGCTT	TGGGACCATG	GTATTTGAAT	GTATTGCTGG	ATATAGGCCCT	660
40	TTTTTGATTC	ATCTGACAGC	ATTACCTGG	CATGAGAAGA	TTAAGAAGAA	GGATCCAAAG	720
	TGTATATTTG	CATGTGAAGA	GATGTCAGGA	GAACTTCGGT	TTAGTAGCCA	TTAACTCTAA	780
	CCAAATAGCC	TTTGTAGTTT	AATAGTAGAA	CCCATGGAAA	ACTGGCTACA	GTGTGATGTT	840
	AATTGGGACC	CTCAGCAGAG	AGGAGGACCT	GTTGACCTTA	CTTTGAAGCA	GCCAAGATGT	900
	TTTGATTAA	TGGATACAT	TTGAATTG	AAGATAGTAC	ACATCCTAAA	TATGACTTCT	960

	GCAAGATAA	TTTCITTTCT	GTTACCACCT	GATGAAAGTC	TTCATTCAC	ACAGTCTCGT	1020
	ATTGAGCGTG	AAACTGGAAT	AAATACTGGT	TCTCAAGAAC	TTCTTTCAGA	GACAGGAATT	1080
	TCTCTGGATC	CTCGGAAC	AGCCTCTCAA	TGTGTTCTAG	ATGGAGTTAG	AGGCTGTGAT	1140
	AGCTATATGG	TTTATTTGTT	TGATAAAAGT	AAAACGTGTAT	ATGAAGGGCC	ATTGTGCTCC	1200
	AGAAGTTTAT	CTGATTGTGT	AAATTATATT	GTACAGGACA	GCAAAATACA	GCTTCCAATT	1260
5	ATACAGCTGC	GTAAGTGTG	GGCTGAAGCA	GTGCACTATG	TGTCTGGACT	AAAAGAAGAC	1320
	TATAGCAGCG	TCTTTCAGG	ACAAAGGGCA	GCAATGTTAA	GTCTTCTTAG	ATATAATGCT	1380
	AACTTAACAA	AAATGAAGAA	CACTTTGATC	TCAGCATCAC	AACAACGTAA	AGCTAAATTG	1440
	GAGTTTTTTC	ACAAAAGCAT	TCAGCTTGAC	TTGGAGAGAT	ACAGCGAGCA	GATGACGTAT	1500
	GGGATATCTT	CAGAAAAAAT	GCTAAAAGCA	TGGAAGAAAA	TGGAAGAAAA	GGCCATCCAC	1560
10	TATGCTGAGG	TTGGTGTCT	TGGATACCTG	GAGGATCAGA	TTATGTCTTT	GCATGCTGAA	1620
	ATCATGGAGC	TACAGAAGAG	CCCTATGGA	AGACGTCAGG	GAGACTTGAT	GGAACTCTCTG	1680
	GAACAGCGTG	CCATTGATCT	ATATAAGCAG	TTAAAACACA	GACCTTCAGA	TCACTCTTAC	1740
	AGTGACAGCA	CAGAGATGGT	GAAAATCATT	GTGCACACTG	TGCAGAGTCA	GGACCGTGTG	1800
	CTCAAGGAGC	TGTTTGTCAT	TTTGAGCAAG	TTGTTGGGCT	GTAAGCAGAA	GATTATTGAT	1860
15	CTACTCCCTA	AGGTGGAAGT	GGCCCTCAGT	AATATCAAAG	AAGCTGACAA	TACTGTCTATG	1920
	TTTCATGAGG	GAAAAGGCCA	GAAAGAAATA	TGGCATCTCC	TTAAAATTGC	CTGTACACAG	1980
	AGTTCTGCC	GTTCCTCTGT	AGGATCCAGT	CTAGAAGGTG	CAGTAACCCC	TCAGACATCA	2040
	GCATGGCTGC	CCCCGACTTC	AGCAGAACAT	GATCAITCTC	TGTCATGTGT	GGTAACCTCT	2100
	CAGATGGGG	AGACTTCAGC	ACAAATGATA	GAAGAAAATT	TGAACTGCCT	TGGCCATTTA	2160
20	AGCACTATTA	TTCATGAGGC	AAATGAGGAA	CAGGGCAATA	GTATGATGAA	TCTTGATTGG	2220
	AGTTGGTTAA	CAGAATGA					2280

## (2) INFORMATION FOR SEQ ID NO:4:

25	(i) SEQUENCE CHARACTERISTICS:
	(A) LENGTH: 745 amino acids
	(B) TYPE: amino acid
	(C) STRANDEDNESS: single
	(D) TOPOLOGY: linear
30	(ii) MOLECULE TYPE: peptide
	(xi) SEQUENCE DESCRIPTION: SEQ ID NO:4:
35	Met Glu Arg Pro Pro Gly Leu Arg Pro Gly Ala Gly Gly Pro Trp Glu
	1 5 10 15
	Met Arg Glu Arg Leu Gly Thr Gly Gly Phe Gly Asn Val Cys Leu Tyr
	20 25 30
	Gln His Arg Glu Leu Asp Leu Lys Ile Ala Ile Lys Ser Cys Arg Leu
40	35 40 45
	Glu Leu Ser Thr Lys Asn Arg Glu Arg Trp Cys His Glu Ile Gln Ile
	50 55 60
	Met Lys Lys Leu Asn His Ala Asn Val Val Lys Ala Cys Asp Val Pro
	65 70 75 80

Glu Glu Leu Asn Ile Leu Ile His Asp Val Pro Leu Leu Ala Met Glu  
                                     85                                    90                                    95  
 Tyr Cys Ser Gly Gly Asp Leu Arg Lys Leu Leu Asn Lys Pro Glu Asn  
                                     100                                    105                                    110  
 5 Cys Cys Gly Leu Lys Glu Ser Gln Ile Leu Ser Leu Leu Ser Asp Ile  
                                     115                                    120                                    125  
 Gly Ser Gly Ile Arg Tyr Leu His Glu Asn Lys Ile Ile His Arg Asp  
                                     130                                    135                                    140  
 Leu Lys Pro Glu Asn Ile Val Leu Gln Asp Val Gly Gly Lys Ile Ile  
                                     145                                    150                                    155                                    160  
 10 His Lys Ile Ile Asp Leu Gly Tyr Ala Lys Asp Val Asp Gln Gly Ser  
                                     165                                    170                                    175  
 Leu Cys Thr Ser Phe Val Gly Thr Leu Gln Tyr Leu Ala Pro Glu Leu  
                                     180                                    185                                    190  
 Phe Glu Asn Lys Pro Tyr Thr Ala Thr Val Asp Tyr Trp Ser Phe Gly  
                                     195                                    200                                    205  
 15 Thr Met Val Phe Glu Cys Ile Ala Gly Tyr Arg Pro Phe Leu His His  
                                     210                                    215                                    220  
 Leu Gln Pro Phe Thr Trp His Glu Lys Ile Lys Lys Lys Asp Pro Lys  
                                     225                                    230                                    235                                    240  
 20 Cys Ile Phe Ala Cys Glu Glu Met Ser Gly Glu Val Arg Phe Ser Ser  
                                     245                                    250                                    255  
 His Leu Pro Gln Pro Asn Ser Leu Cys Ser Leu Ile Val Glu Pro Met  
                                     260                                    265                                    270  
 Glu Asn Trp Leu Gln Leu Met Leu Asn Trp Asp Pro Gln Gln Arg Gly  
                                     275                                    280                                    285  
 25 Gly Pro Val Asp Leu Thr Leu Lys Gln Pro Arg Cys Phe Val Leu Met  
                                     290                                    295                                    300  
 Asp His Ile Leu Asn Leu Lys Ile Val His Ile Leu Asn Met Thr Ser  
                                     305                                    310                                    315                                    320  
 30 Ala Lys Ile Ile Ser Phe Leu Leu Pro Pro Asp Glu Ser Leu His Ser  
                                     325                                    330                                    335  
 Leu Gln Ser Arg Ile Glu Arg Glu Thr Gly Ile Asn Thr Gly Ser Gln  
                                     340                                    345                                    350  
 Glu Leu Leu Ser Glu Thr Gly Ile Ser Leu Asp Pro Arg Lys Pro Ala  
                                     355                                    360                                    365  
 35 Ser Gln Cys Val Leu Asp Gly Val Arg Gly Cys Asp Ser Tyr Met Val  
                                     370                                    375                                    380  
 Tyr Leu Phe Asp Lys Ser Lys Thr Val Tyr Glu Gly Pro Phe Ala Ser  
                                     385                                    390                                    395                                    400  
 40 Arg Ser Leu Ser Asp Cys Val Asn Tyr Ile Val Gln Asp Ser Lys Ile  
                                     405                                    410                                    415  
 Gln Leu Pro Ile Ile Gln Leu Arg Lys Val Trp Ala Glu Ala Val His  
                                     420                                    425                                    430  
 Tyr Val Ser Gly Leu Lys Glu Asp Tyr Ser Arg Leu Phe Gln Gly Gln

	435		440		445	
	Arg	Ala	Ala	Met	Leu	Ser
	450		455		460	
	Met	Lys	Asn	Thr	Leu	Ile
	465		470		475	
5	Glu	Phe	Phe	His	Lys	Ser
	485		490		495	
	Gln	Met	Thr	Tyr	Gly	Ile
	500		505		510	
	Glu	Met	Glu	Glu	Lys	Ala
10	515		520		525	
	Tyr	Leu	Glu	Asp	Gln	Ile
	530		535		540	
	Gln	Lys	Ser	Pro	Tyr	Gly
	545		550		555	
15	Glu	Gln	Arg	Ala	Ile	Asp
	565		570		575	
	Asp	His	Ser	Tyr	Ser	Asp
	580		585		590	
	Thr	Val	Gln	Ser	Gln	Asp
20	595		600		605	
	Ser	Lys	Leu	Leu	Gly	Cys
	610		615		620	
	Val	Glu	Val	Ala	Leu	Ser
	625		630		635	
25	Phe	Met	Gln	Gly	Lys	Arg
	645		650		655	
	Ala	Cys	Thr	Gln	Ser	Ser
	660		665		670	
	Gly	Ala	Val	Thr	Pro	Gln
30	675		680		685	
	Glu	His	Asp	His	Ser	Leu
	690		695		700	
	Thr	Ser	Ala	Gln	Met	Ile
	705		710		715	
35	Ser	Thr	Ile	Ile	His	Glu
	725		730		735	
	Asn	Leu	Asp	Trp	Ser	Trp
	740		745			

40 (2) INFORMATION FOR SEQ ID NO:5:

(1) SEQUENCE CHARACTERISTICS:

(A) LENGTH: 2146 base pairs

(B) TYPE: nucleic acid

(C) STRANDEDNESS: double  
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: cDNA

5	(xi) SEQUENCE DESCRIPTION: SEQ ID NO:5:	
	GTACCAGCAT CGGAACTTG ATCTCAAAT AGCAATTAAAG TCTGTGCGCC TAGAGCTAAG	60
	TACCAAAAC AGAGAAGCAT GGTGCCATGA AATCCAGATT ATGAAGAAGT TGAACCATGC	120
	CAATGTTGTA AAGGCGTGTG ATGTTCCCTGA AGAATTGAAT ATTTTGATTC ATGATGTGCC	180
	TCTTCTAGCA ATGGAATACT GTTCTGAGAG AGATCTCCGA AAGCTGCTCA ACAAAACCAGA	240
10	AAATGTGTGT GGACTTAAAG AAAGCCAGAT ACTTCTTTA CTAAGTGATA TAGGGTCTGG	300
	GATTTCATAT TTGCATGAAA ACAAAATTAT ACATCGAGAT CTAACACCTG AAAACATAGT	360
	TCTTCAGATG GTTGGTGGAA AGATAATACA TAAAAATAAT GATCTGGGAT ATGCCAAGA	420
	TGTTGATCAA GGAAGTCTGT GTACATCTTT TGTGGGAACA CTGCAGTATC TGGCCCCAGA	480
	GCTCTTTGAG AATAAGCCTT ACACAGCCAC TGTGTATTAT TGGAGCTTTG GGACCATGGT	540
15	ATTTGAATGT ATTGCTGGAT ATAGGCCTTT TTTGCATCAT CTGCAGCAT TTAACCTGGCA	600
	TGAGAAGATT AAGAGTGAAG ATCCAAAGTG TATAATTGCA TGTGAAGAGA GTGCAGGAGA	660
	AGTTCGGTIT AGTAGCCATT TACCTCAACC AAATAGCCTT TGTAGTTTAA TAGTAGAACC	720
	CATGGAAGAC TGGCTACAGT TGATGTTGAA TTGGGACCTT CAGCAGAGAG GAGGACCTGT	780
	TGACCTTACT TTGAAGCAGC CAAGATGTTT TGTATTAAATG GATCACATT TGAATTTGAA	840
20	GATAGTACAC ATCTCAAATA TGACTTCTGC AAAATAATT TCTTTTCTGT TACCACCTGA	900
	TGAAGTCTT CATCTACTAC AGTCTCGTAT TGAGCGTGAA ACTGGAATAA ATACTGGTTC	960
	TCAAGAACTT CTTTCAGAGA CAGGAATTTT TCTGGATCCT CGGAAACAGC CCTCTCAATG	1020
	TGTTCTAGAT GGAGTTAGAG GCTGTGATAG CTATATGGTT TATTGTGTTG ATAAAGTAA	1080
	AACTGTATAT GAAGGGCCAT TTGCTTCCAG AAGTTTATCT GATTGTGTAA ATTATATTGT	1140
25	ACAGGACAGC AAAATACAGC TTCCAATTAT ACAGCTGCGT AAAGTGTGGG CTGAAGCAGT	1200
	GCACTATGTG TCTGACTAA AAGAAGACTA TAGCAGGCTC TTTCAGGGAC AAAGGGCAGC	1260
	AATGTTAAGT CTCTTAGAT ATAATGCTAA CTTAACAATA ATGAAGACA CTTTGTATCTC	1320
	AGCATCACAA CAATGAAAG CTAATTTGGA GTTTTTTAC AAAAGCATT AGCTTGACTT	1380
	GGAGAGATAC AGCGAGCAGA TGACGTATGG GATATCTTCA GAAAAATGC TAAAGCATG	1440
30	GAAAGAAATG GAAGAAAGG CCATCCACTA TGCTGAGGTT GGTGTCAATT GATACCTGGA	1500
	GGATCAGATT ATGTCTTTGC ATGTGAAAT CATGGAGCTA CAGAAGAGCC CCTTGTGAAG	1560
	ACGTACAGGA GACTTGATGG AATCTCTGGA ACAGCGTGCC ATGATCTAT ATAAGCAGTT	1620
	AAAACACAGA CCTTCAGATC ACTCTACAG TGACAGCACA GAGATGGTGA AAATCATTGT	1680
	GCACACTGTG CAGACTCAGC ACCGTGTGCT CAAGGAGCGT TTTGGTCAAT TGAGCAAGTT	1740
35	GTGGGCTGT AAGCAGAAGA TTAATTGATCT ACTCCCTAAG GTGGAAGTGG CCCTCAGTAA	1800
	TATCAAGAA GCTGACAATA CTGTGATGTT CATGCAGGGA AAAAGGCAGA AAGAAATATG	1860
	GCATCTCCTT AAAATTGCCT GTACACAGAG TTCTGCCCGC TCTCTGTAG GATCCAGTCT	1920
	AGACAGTGCA GTAACCCCTC AAGCATACG ATGGCTGGCC CCGACTTAG CAGAACATGA	1980
	TCATTCTCTG TCATGTGTGG TAACTCCTCA AGATGGGGAG ACTTCAGCAC AAATGTATAGA	2040
40	AGAAAATTIG AACTGCTTGG GCCATTAAAG CACTATTATT CATGAGGCAA ATGAGGAACA	2100
	GGCAATAGT ATGATGAATC TTGATTGGAG TTGGTTAACA GAATGA	2146